Influence of animal manure application on the chemical structures of soil organic matter as investigated by advanced solid-state NMR and FT-IR spectroscopy

Jingdong Mao [a,*], Dan C. Olk [b], Xiaowen Fang [c], Zhongqi He [d], Klaus Schmidt-Rohr [c]

[a] Department of Chemistry and Biochemistry, Old Dominion University, 4541 Hampton Blvd, Norfolk, VA 23529, United States
[b] USDA-ARS, National Soil Tilth Laboratory, 2110 University Blvd., Ames, IA 50011, United States
[c] Department of Chemistry, Gilman Hall, Iowa State University, Ames, IA 50010, United States
[d] USDA-ARS, New England Plant, Soil, and Water Laboratory, Orono, ME 04469, United States

ARTICLE INFO

ABSTRACT

Annual application of cattle manure to a farmer's field in eastern Nebraska for 5 yr caused improved soil N and P supply and increased corn (*Zea mays* L.) yield in less productive portions of the field compared to another field treatment receiving the same amount of N as inorganic fertilizer. As a first step toward identifying the soil processes that led to these changes, the effects of manure addition on the chemical nature of soil organic matter were investigated by extracting two humic fractions—mobile humic acid (MHA) and calcium humate (CaHA)—from the field soil for subsequent analysis by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy and Fourier Transform-infrared (FT-IR) spectroscopy. Functional groups were selectively identified and quantified through NMR techniques that included quantitative direct polarization, cross polarization/total suppression of sidebands, ^{1}H-^{13}C heteronuclear correlation NMR, ^{13}C chemical shift anisotropy filtering, CH-signal selection, and other spectral-editing techniques. The CaHAs exhibited high aromaticity and line shapes typical of Mollisols rich in oxidized charcoal, while the MHAs had lower aromaticity and greater contributions from lignin aromatic ethers. Results show that the chemical nature of each humic fraction did not differ significantly between an unfertilized control and the treatment receiving N as inorganic fertilizer. The FT-IR results were consistent with all these findings. The NMR results further showed that the CaHA fraction from the manure treatment was clearly depleted in aromatic rings and enriched in nonpolar alkyl compounds, most likely fatty acids, compared to the other two fertilizer treatments. Selective spectra further show strong indications of enhanced peptide contributions in the manure CaHA, consistent with the improved soil N supply associated with the manure treatment. The peptide enrichment was partially masked in unselective spectra by a simultaneous decrease in overlapping signals for OCH$_3$ and COO groups that are associated with aromatic components, demonstrating the improved accuracy of the selective spectral-editing NMR techniques.

1. Introduction

The benefits of animal manure application to soil organic matter (SOM) quantity, nutrient availability, soil aggregation, and other soil functions are well known (Olsen, 1986; Paul et al., 1997; Hatfield and Stewart, 1998). For example, in a 5-yr study of a Nebraska farmer's field planted to continuous corn (*Zea mays* L.), annual application of fresh (i.e. not composted) beef cattle manure improved grain yield in lower-yielding areas of the field compared to (i) application of an N-equivalent amount of mineral fertilizer, and (ii) a control treatment lacking fertilizer input (B. Eghball, pers. commun, 2001). Stepwise multiple regression identified levels of available soil N and soil P as the best predictors of the yield increase, suggesting some benefit of the manure application to nutrient supply beyond the amounts of immediately available nutrients in the manure.

Previous studies indicated that some manure-bound nutrients are gradually released from the organic component of the manure (Van Faassen and Van Dijk, 1987; Bouldin and Klausner, 1998). The release rates of these organic nutrients can conceivably be affected by the chemical nature of the manure and of the SOM that forms from the manure. Compared to the vast literature on the cycling of rapidly available nutrients in manure, though, less information is available regarding longer-term effects of manuring on the chemical nature of SOM and the potential repercussions for nutrient cycling.

A limited number of studies used various instrumental technologies to investigate the chemical nature of SOM in soil amended with manure. Using solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy and amino acid analysis, Campbell et al. (1986) concluded that animal manure application enabled an enrichment of amino acids and amino sugars in labile SOM, thereby promoting an

---

* Corresponding author. Tel.: +1 737 683 6874; fax: +1 757 683 4628.
 E-mail address: jmao@odu.edu (J. Mao).

observed increase in crop N supply. Brunetti et al. (2007) used NMR spectroscopy to show that a one-time application of liquid swine manure resulted in weak signals for O- and N-substituted aromatic C and a slight increase in methoxy-C for a humic acid fraction. After annual field applications of swine manure slurry for 3 to 4 yr, Plaza et al. (2002) and Senesi et al. (2007) used elemental composition, Fourier transform-infrared (FT-IR) spectroscopy, electron spin resonance spectroscopy and fluorescence spectroscopy to conclude that N-rich and other aliphatic components of the manure were initially incorporated into a soil humic fraction, but long-term mineralization caused a general decrease in their enrichment despite continued manure applications, so that only the most recalcitrant manure components, such as S-containing, phenolic, and aliphatic molecules accumulated long-term in the humic fraction. Application of farmyard manure led to an accumulation of fatty acids as measured by gas chromatography (Jandl et al., 2005). Randall et al. (1995) used solid-state ^{13}C cross-polarization (CP) NMR to find that mineral fertilizer and manure differed little in their effects on the chemical composition of SOM associated with clay- and silt-sized fractions. The authors concluded that the chemical composition of SOM was determined primarily by the interaction between the organisms responsible for decomposition and the mineral soil matrix rather than by the nature of substrate input. Using FT-IR spectroscopy, Wander and Traina (1996) associated an organically managed crop rotation that included manure application with more "reactive" SOM, which was defined by the ratios of functional groups in SOM fractions.

Solid-state NMR spectroscopy is a powerful tool to study the structure of SOM because it is nondestructive, it can detect insoluble organic matter, and it can provide comprehensive structural information (Preston, 1996). Nearly all of the many SOM studies involving ^{13}C solid-state NMR used routine techniques of ^{13}C cross polarization/magic angle spinning (CP/MAS). The resulting broad spectra of SOM provided only limited information on specific structures.

In recent years we have developed, adapted, and applied many new advanced solid-state NMR techniques for characterizing complex organic matter in plants, soils, water, and sediments (Levin et al., 2007; Mao et al., 2000, 2003, 2006, 2007a,b,c; Mao et al., 2008; Mao and Schmidt-Rohr, 2005; Schmidt-Rohr and Mao, 2002a,b,c, 2004). These techniques identify functional groups, their connectivities, as well as heterogeneities and domains in natural organic matter; they include CH spectral-editing (Schmidt-Rohr and Mao, 2002a), ^{13}C{^{14}N} NMR identifying C bonded to N (Schmidt-Rohr and Mao, 2002c), ^{1}H–^{13}C heteronuclear correlation, recoupled long-range dipolar dephasing (Mao and Schmidt-Rohr, 2003), and ^{13}C chemical-shift anisotropy filtering (Mao and Schmidt-Rohr, 2004b). Especially, the new spectral-editing techniques can selectively retain certain peaks and eliminate others, clearly revealing specific functional groups.

Another nondestructive technique, infrared spectroscopy, usually in the form of FT-IR spectroscopy, is a relatively important yet simple tool for the characterization of organic matter (Giusquiani et al., 1998; Fan et al., 2000; Olk et al., 2000; Tan, 2003; He et al., 2006b). This technique provides detailed information about the oxygen-containing groups present in the complex molecules, since polar groups usually show very intense infrared signals (Francioso et al., 1996). Recently, He et al. (2006a, 2007) demonstrated that vibration bands of P–O bonds of phosphate compounds appear in the region at 1100–1000 cm^{-1}. He et al. (2006b) correlated the intensity of this absorption band with the P content of six humic and fulvic acid samples, suggesting that the characteristic absorbance at 1100–1000 cm^{-1} may provide a simple and rapid diagnosis for the P content of purified humic substances.

The objective of this study is to use state-of-the-art solid-state NMR techniques and FT-IR to study the chemical structures of two SOM fractions, namely the mobile humic acid (MHA) and the calcium humic acid (CaHA), that were chemically extracted from soils of the manure, mineral fertilizer, and unfertilized treatments in the 5-year

Nebraska study of continuous corn. These two fractions differed in their chemical nature, responsiveness in quantity and chemical nature to crop management, and involvement in N cycling for tropical rice (*Orzya sativa* L.) soils, with the MHA being more labile in every comparison (Mahieu et al., 2002; Olk et al., 2007). In one study of Midwestern agriculture, various corn-based rotations in Nebraska affected both fractions in quantity and nutrient cycling as measured by ^{13}C and ^{15}N isotope contents (Legorreta-Padilla, 2005). A limited number of other studies also found the MHA fraction was younger or more labile than the CaHA fraction (Olk, 2006). Otherwise this fractionation procedure has not yet been extensively evaluated in upland crop soils. Analysis of their chemical natures in this study is the first step toward the evaluation of manure effects on SOM in the Nebraska corn field.

2. Materials and methods

2.1. Soil samples

The experiment was conducted from autumn 1997 through 2002 on a private farm in Hamilton County near Phillips, Nebraska, on an irrigated field with a continuous corn system. Fertilizer treatments were established in the field with four replicates. They included application of beef cattle feedlot manure, inorganic N fertilizer, and an unfertilized control. The manure was applied in the autumns of 1997 to 2001 at an N rate that would provide for a corn production level of 10.7 Mg ha^{-1} (Hergert et al., 1995) in compliance with the landowner's request. Starter fertilizer as liquid ammonium polyphosphate was applied at planting to all treatments at a rate of 10 kg N ha^{-1} in each year. Anhydrous ammonia was side dressed to fertilizer strips on 22 June 1998 and 22 June 1999 at a rate of 202 kg N ha^{-1}. In 2000 to 2002, anhydrous ammonia was applied at planting time (~ mid May) at a rate of 210 kg N ha^{-1}. The unfertilized control plots were divided into two rotating strips. Synthetic fertilizer at a rate similar to the inorganic fertilizer treatment was applied to one half, and the other half was not fertilized. Each year the strip that did not receive any N input was considered the unfertilized control. This was done to avoid severe N deficiency in the unfertilized plots on this mostly sandy soil.

Fertilizer treatments were divided into sections based on soil organic C levels, and the soils that were selected for this study were taken from the section with organic C levels >14 g kg^{-1}, which was mapped as the Hord silt loam (fine silty, mixed, mesic Cumulic Haplustolls). The topsoils (0–15 cm) were sampled in June 2002. Soil samples were air-dried in a greenhouse, hand-ground to pass through an 8-mm mesh, and analyzed for total C and total N by automated combustion.

2.2. Humic acid extraction

The MHA and CaHA fractions were extracted with modifications of the procedure by Olk et al. (1996). Briefly, 35-g batches of air-dry soil were shaken in 350 mL of 0.25 M NaOH under N$_2$ gas for 20 h. After centrifuging, the supernatant was decanted and acidified to pH 2.0 to precipitate the MHA fraction. The soil was incubated again overnight in NaOH solution for a second extraction of the MHA to ensure thorough extraction of this fraction. To remove soil Ca, the soil was then washed with 0.2 M HCl followed by centrifuging and disposal of the supernatant, and these steps were repeated until the pH of the supernatant remained below 1.0. The soil was similarly washed in water to remove excess HCl until the supernatant pH rose above 2.0. Soil organic matter that had been bound to Ca was then extracted by shaking the soil overnight again in NaOH, and subsequent acidification of the supernatant caused precipitation of the CaHA fraction. Extraction of the CaHA fraction was repeated during a second overnight incubation in NaOH, as with the MHA fraction. To remove soil contaminants, both fractions were solubilized in a 0.05 M KCl/0.25 M KOH

solution (Swift, 1996) and centrifuged, and the supernatants were decanted and acidified to reprecipitate the fractions. Then the fractions were shaken for 24 h in a 0.5% HCl/HF solution to remove any persisting inorganic contaminants. To H-saturate the fractions and remove other cations, the fractions were dialyzed for 2 d against successively weaker HCl solutions and for a third day against water. Finally the fractions were dried by lyophilizing. The C and N concentrations of the MHA and CaHA fractions were determined by automated combustion. The results of soil C concentration, C and N concentrations of two extracted humic fractions, and C content of the soil extracted as either humic fraction are listed in Table 1. The dried MHA and CaHA fractions contained 520 to 540 g C kg^{-1}, indicating a pure humic material with no soil or salt contamination. The MHA contained about 50 g N kg^{-1} for all fertilizer treatments. The CaHA fraction contained *about* 40 g N kg^{-1} for the unfertilized and inorganic fertilizer treatments but 50 g N kg^{-1} for the manured treatment. The MHA fraction contained 14 to 18% of total soil C, and the CaHA fraction contained 8 to 11% of total soil C.

2.3. NMR spectroscopy

All the experiments were performed on a Bruker DSX400 spectrometer at 100 MHz for ^{13}C, using MAS. Most experiments were performed with 7-mm sample rotors for best sensitivity, in a double-resonance probe head. An exception was the high-speed experiments at 14 kHz MAS, acquired using a 4-mm double-resonance probe head.

2.3.1. High-speed quantitative ^{13}C DP/MAS NMR
This technique provides quantitative structural information. Quantitative ^{13}C Direct Polarization/Magic Angle Spinning (DP/MAS) was run at a spinning speed of 14 kHz. The 90° ^{13}C pulse-length was 4 µs. Recycle delays were tested by the Cross Polarization/Spin Lattice Relaxation Time–Total Sideband Suppression (CP/T$_1$–TOSS) technique to ensure that all carbon sites were fully relaxed (Mao et al., 2000). The details of this technique have been described elsewhere (Mao et al., 2000).

2.3.2. ^{13}C CP/TOSS and ^{13}C CP/TOSS with dipolar dephasing
Qualitative composition information was obtained with good sensitivity by ^{13}C CP/TOSS. NMR experiments at a spinning speed of 6.5 kHz and a CP time of 1 ms, with a ^{1}H 90° pulse-length of 4 µs and a recycle delay of 1 s. Four-pulse total suppression of sidebands (TOSS) (Dixon, 1982) was employed before detection, and two-pulse phase-modulated (TPPM) decoupling was applied for optimum resolution. The corresponding subspectrum with signals of nonprotonated carbons and mobile groups such as rotating CH$_3$ was obtained by ^{13}C CP/TOSS combined with 40-µs dipolar dephasing.

2.3.3. ^{13}C chemical-shift-anisotropy filter
In order to separate the signals of anomeric carbons (O–C–O) from those of aromatic carbons, both of which may resonate between 120 and 90 ppm, the aromatic-carbon signals were selectively suppressed by a five-pulse ^{13}C chemical-shift-anisotropy (CSA) filter with a CSA-filter time of 35 µs (Mao and Schmidt-Rohr, 2004a). In order to select

the signals of nonprotonated O–C–O (ketal) carbons, which may extend to 120 ppm, this CSA filter was combined with a dipolar dephasing time of 40 µs. In a complementary experiment, selective spectra of protonated anomerics (O–CH–O, acetals) were obtained by CSA filtering after short CP. The details of this technique have been described elsewhere (Mao and Schmidt-Rohr, 2004a).

2.3.4. Spectral-editing of immobile CH$_2$+CH
The combined spectrum of these chemical groups can be obtained with good sensitivity in a simple spectral-editing experiment. First, a CP/TOSS spectrum is recorded using a short CP of 50 µs. It shows predominantly protonated carbons in immobile segments, but residual peaks of quaternary carbons result from two-bond magnetization transfer. Second, a CP/TOSS spectrum is recorded using a short CP of 50 µs and 40 µs dipolar dephasing. It contains only the residual signals of quaternary carbons or mobile segments (including CH$_3$ groups with >50% efficiency). The difference of the two spectra is the spectrum of immobile CH$_2$ and CH carbons, with a small CH$_3$ contribution.

2.3.5. CH spectral-editing
For CH (methine) selection, a robust method based on C–H multiple-quantum coherence (Schmidt-Rohr and Mao, 2002a) was used at 5.787 kHz MAS. CH-group multiple-quantum coherence is not dephased by the spin-pair CH dipolar coupling while CH$_2$ coherence is dephased by dipolar coupling of the carbon to the two protons. The first of a pair of recorded spectra contains signals of CH, as well as residual quaternary-carbon and CH$_3$ peaks that are removed by taking the difference with a second spectrum acquired with the same pulse sequence except for additional 40-µs dipolar dephasing before detection. The recycle delay was 1 s, and the total measuring time for each spectrum was 13 h.

2.3.6. ^{1}H–^{13}C Heteronuclear Correlation (HETCOR) NMR
Two-dimensional (2D) HETCOR NMR experiments (Mao and Schmidt-Rohr, 2006) were performed at a spinning speed of 6.5 kHz. Lee–Goldburg cross polarization (LGCP) of 0.5 ms was used to suppress ^{1}H–^{1}H spin diffusion during polarization transfer. The spectrum shows mostly one- and two-bond ^{1}H–^{13}C connectivities.

2.4. Fourier Transform-Infrared Spectroscopy

The FT-IR spectra were obtained from discs containing about 1 mg sample and 100 mg KBr. The spectra were recorded in the 450–4000 cm^{-1} range on a PerkinElmer Spectrum One FT-IR Spectrophotometer (Perkin-Elmer Instruments, Norwalk, CT). Each sample was scanned 24 times with a resolution of 2 cm^{-1}. All spectra were normalized.

3. Results

3.1. Overall NMR analysis

For all six samples, Fig. 1 shows the ^{13}C DP/MAS spectra, which can provide quantitative structural information. The assignments and the percentages of different functional groups are listed in Table 2.

Table 1
Soil C concentration, C and N concentrations of two extracted humic fractions, and C content of the soil extracted as either humic fraction

Fertilizer treatment	Soil C	Mobile humic acid (MHA) fraction				Calcium humate (CaHA) fraction			
		C concentration	N concentration	MHA-C content	% of soil C	C concentration	N concentration	CaHA-C content	% of soil C
	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1} soil		g kg^{-1}	g kg^{-1}	g kg^{-1} soil	
Unfertilized	20.0	540	49	3.2	16	530	39	2.2	11
Inorganic fertilizer	19.4	530	51	2.8	14	520	41	1.6	8
Manure	26.1	540	52	4.6	18	540	50	2.5	10
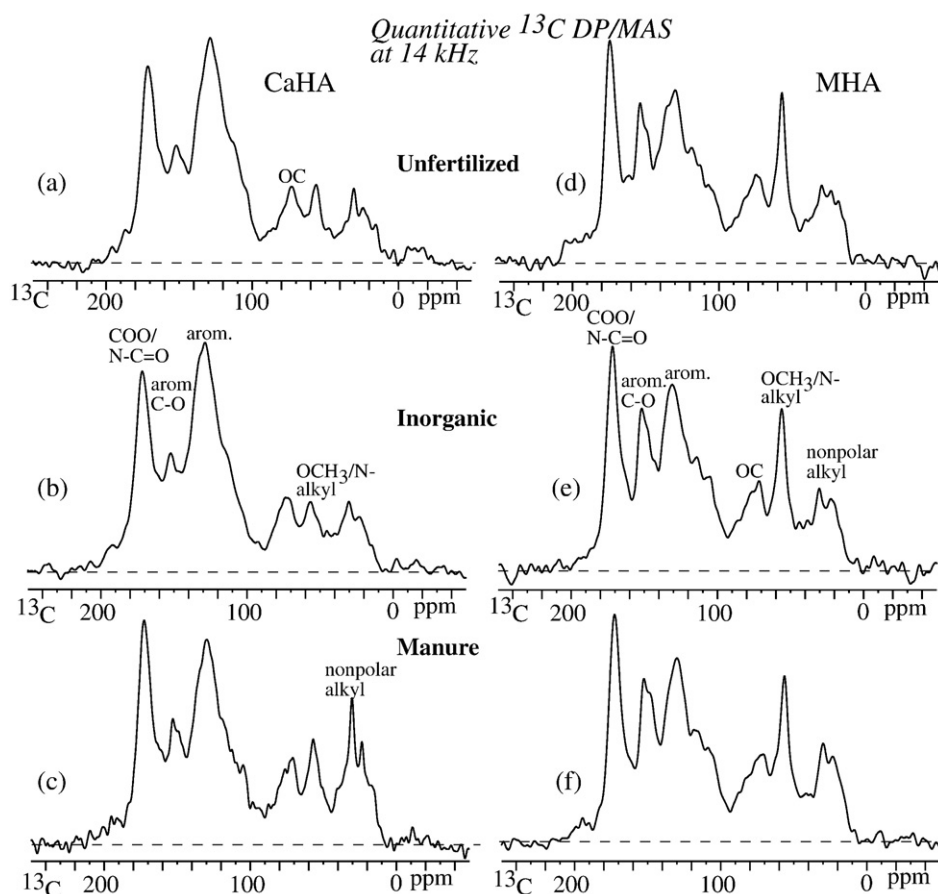
Fig. 1. Quantitative ^{13}C DP/MAS NMR at a spinning speed of 14 kHz of (a) Calcium HA with no fertilization, (b) Calcium HA with inorganic fertilization, (c) Calcium HA with manure application, (d) Mobile HA with no fertilization, (e) Mobile HA with inorganic fertilizer, and (f) Mobile HA with manure application. Numbers of scans and recycle delays: (a) 460 scans, 60 s; (b) 928 scans, 50 s; (c) 916 scans, 30 s; (d) 1536 scans, 50 s; (e) 540 scans, 100 s; and (f) 992 scans, 50 s.

Roughly, the assignments are as follows (Mao et al., 2000): 0–44 ppm, nonpolar alkyl; 44–64 ppm, NCH and OCH$_3$; 64–93 ppm, carbohydrate C; 93–110 ppm, O–C–O anomerics; 93–143 ppm, aromatic C; 143–159 ppm, aromatic C–O; 159–190 ppm, COO and N–C=O; and 190–220 ppm, ketone, quinone, or aldehyde C.

The proportions of total spectral area differed noticeably between the CaHA and MHA fractions for several functional groups. The CaHA contained more aromatic C and COO/N–C=O but less aromatic C–O and N-alkyl/methoxy C than did the MHA (Table 2). We did not find clear differences between the unfertilized MHA, inorganic MHA, and manure MHA, except that the nonpolar alkyl peak appears taller and sharper in the manure MHA than the other two MHAs, which could be due to more crystalline poly(methylene) (Hu et al., 2000). Nevertheless, the spectral proportion of nonpolar alkyl differed little among the three MHAs (Table 2).

3.2. Manure CaHA vs. other CaHAs

While there were no notable differences between the unfertilized CaHA and inorganic CaHA, the manure CaHA was distinctly different. For instance, it had more nonpolar alkyl C and less aromatic C than did the other two CaHA samples. When the spectral proportions are combined for the two alkyl regions of 0–44 ppm and 44–64 ppm, the summed proportion increased across treatments from unfertilized to inorganic fertilizer to manure from 15.1% to 15.8% to 21.7%, respectively, for the CaHA and from 21.9% to 22.6% to 23.6%, respectively, for the MHA. When the spectral proportions are combined for the two aromatic regions of 93–143 ppm and 143–159 ppm, the summed proportion decreased across treatments from unfertilized to inorganic fertilizer to manure from 52.5% to 50.4% to 44.7%, respectively, for the CaHA and from 47.2% to 46.9% to 44.7%, respectively, for the MHA.

Table 2
Percentages of total spectral area assigned to different functional groups obtained by quantitative DP/MAS ^{13}C NMR for the mobile humic acid (MHA) and calcium humate (CaHA) fractions that were extracted from three fertilizer treatments

ppm	190–220	159–190	143–159	93–143	93–110	64–93	44–64	0–44
Assignments	Ketones, aldehydes	COO/N–C=O	Arom. C–O	Aromatics	Alkyl O–C–O	O-alkyl	N-alkyl, methoxyl	Nonpolar Alkyl
Unfertilized MHA	2	15.9	14.7	32.5	2.2	10.9	11.0	10.9
Inorganic MHA	1.0	17.7	14.5	32.4	2.0	9.8	10.8	11.8
Manure MHA	1.0	17.7	14.6	30.1	2.2	10.8	11.8	11.8
Unfertilized CaHA	1.0	20.1	10.7	41.8	2.3	9.1	6.0	9.1
Inorganic CaHA	2.0	20.7	9.4	41.0	2.3	8.9	6.9	8.9
Manure CaHA	2.0	20.6	9.3	35.4	2.3	8.8	7.9	13.8

Sidebands are corrected. Anomeric Cs are separated from aromatic Cs based on the ^{13}C CSA-filtered spectra.
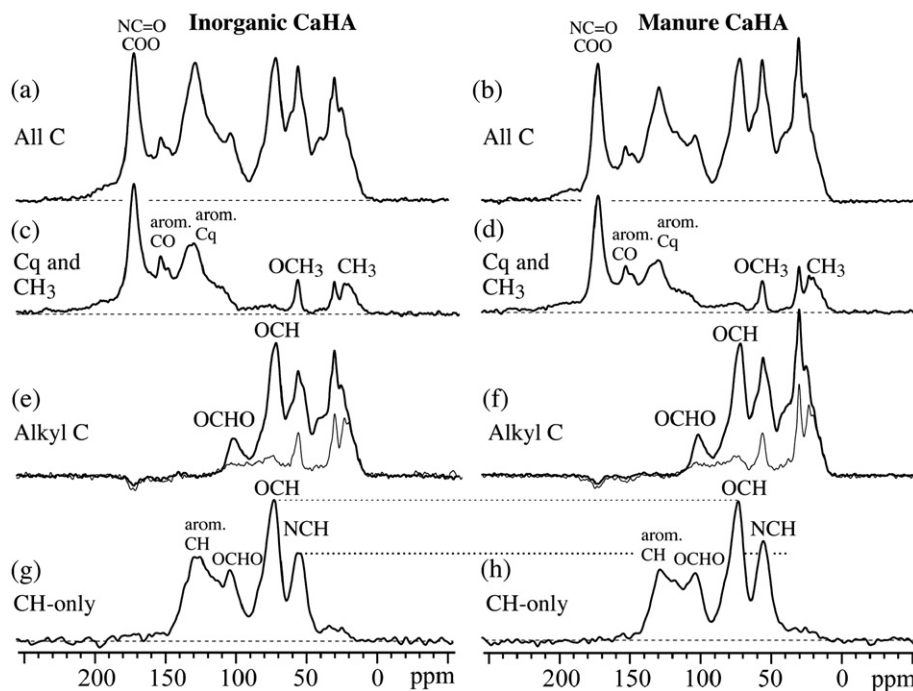
Fig. 2. Spectral editing for identification of functional groups in ^{13}C NMR of calcium HAs from soil fertilized by inorganic fertilizer (left column) and manure (right column). (a, b) Unselective CP/TOSS spectra for reference, with a contact time of 1 ms at a spinning speed of 6.5 kHz. (c, d) Corresponding dipolar-dephased CP/TOSS spectra showing nonprotonated carbons and mobile segments such as CH$_3$, acquired after 40-μs of decoupling gated off. (e, f) Selection of sp^3-hybridized carbon signals by a chemical-shift anisotropy filter, which in particular identifies OCO carbons, near 102 ppm, which are typical of sugar rings. CSA filter time: 35 μs, other parameters as in (a). Thin line: Corresponding selection of nonprotonated or mobile sp^3-hybridized carbon signals by a 35-μs CSA filter and 40-μs dipolar dephasing. Scaled up vertically by 1.61 to correct for dephasing of CH$_3$ groups. (g, h) Spectra of CH (methine) carbons. All spectra were scaled to equivalent height of the OCH peak.

In order to better characterize these effects of manure application on humic structure, we selected the manure and inorganic CaHA samples for more detailed spectral-editing NMR studies. Fig. 2 shows a series of ^{13}C CP/MAS NMR spectra for inorganic and manure CaHAs that were acquired with suitably designed radio-frequency pulse sequences to select subspectra of specific types of chemical groups. Fig. 2(a) and (b) are ^{13}C CP/TOSS spectra, which show qualitative structural information and are used primarily as reference spectra for the selective subspectra. The corresponding CP/TOSS spectra after 40-μs of dipolar dephasing (Fig. 2(c) and (d)) exhibit solely signals of nonprotonated carbons and mobile groups, including rotating CH$_3$ groups, which have a reduced C–H dipolar coupling due to their fast motion. For both CaHAs, these spectra show merely small signals from highly mobile CH$_3$ (10–24 ppm) and (CH$_2$)$_n$ (around 30 ppm) components, and stronger signals from COO/NC=O groups (173 ppm). In addition, nonprotonated aromatic carbons in multiple oxygen-substituted rings are detected around 155 ppm and 110 ppm. Clear OCH$_3$ signals are distinct around 55 ppm. These three types of bands are characteristic of lignin. Nonprotonated aromatic signals are also observed around 130 ppm and a small, broad band around 65–95 ppm arises from OC$_q$. There are more nonprotonated aromatics (signals around 130 ppm) in the inorganic CaHA than in the manure CaHA.

The CP/TOSS spectra after a ^{13}C CSA filter of 35 μs, which exhibit only sp^3-carbon signals, are displayed in Fig. 2(e) and (f). In particular, this technique separates overlapping anomerics (O–C–O) from aromatics between 90 and 120 ppm. A clear O–C–O band is displayed in this region. The ratios of O–C–O to alkyl OC for both CaHAs were near 1:5, indicating the dominance of 6-membered ring carbohydrates for CaHAs. Note that the alkyl OC region between 60 and 90 ppm includes OC$_q$, OCH and OCH$_2$, i.e. the rest of sugar carbons except O–C–O anomerics which are the only carbons connected to two oxygens in sugar rings. The combination of this filter technique with dipolar dephasing leads to subspectra of solely nonprotonated or mobile sp^3-carbon sites (thin lines in Fig. 2(e) and (f)). Only small

amounts of quaternary O–C$_q$–O groups are present in either CaHAs, showing that alkyl OCO groups are dominantly protonated for the two CaHAs. Fig. 2(g) and (h) show the spectra of CH (methine) groups in the CaHAs, acquired using dipolar DEPT. A significant (~13%) increase in the NCH signal, relative to the OCH peak, is observed as a result of the manure application.

3.3. MHA vs. CaHA composition

Fig. 1 indicates that three MHAs are structurally quite similar, and so are the CaHAs except for those differences in the manure CaHA discussed in the preceding section. Because of these similarities, a comparison of the structures of the CaHA and MHA fractions can be restricted to one sample of each fraction. We selected the inorganic CaHA and MHA samples for this purpose. Similar to Fig. 2, Fig. 3 shows a series of ^{13}C CP/MAS NMR spectral-editing spectra for inorganic MHA and CaHA. Fig. 3(a) and (b) are reference ^{13}C CP/TOSS spectra. For both the MHA and CaHA, the corresponding CP/TOSS spectra after 40-μs of dipolar dephasing (Fig. 3(c) and (d)) show highly mobile CH$_3$ and (CH$_2$)$_n$, OCH$_3$, OC$_q$, nonprotonated aromatic carbons, COO/NC=O groups, and ketones. The three types of bands characteristic of lignin (150, 110 and 55 ppm) were more prominent in the MHA than in the CaHA. There are more nonprotonated aromatics around 130 ppm in the CaHA than in the MHA.

The CP/TOSS spectra after a ^{13}C CSA filter are displayed in Fig. 3(e) and (f). The ratios of O–C–O to OC for the MHA and CaHA were 1:4 and 1:5, respectively, indicating the existence of 5-member ring carbohydrates in the MHA and 6-member ring carbohydrates in the CaHA. The combination of this filter technique with dipolar dephasing (Fig. 3(g) and (h)) shows that only small amounts of quaternary O–C$_q$–O groups are present in either the MHA or CaHA. Compared with the spectra after dipolar dephasing, lignin-derived OCH$_3$ peaks around 55 ppm are significantly reduced after the ^{13}C CSA filter due to the relatively larger CSAs of OCH$_3$ compared with those of other alkyl carbons. Still, the OCH$_3$ peak of the MHA is larger than that of the CaHA. The
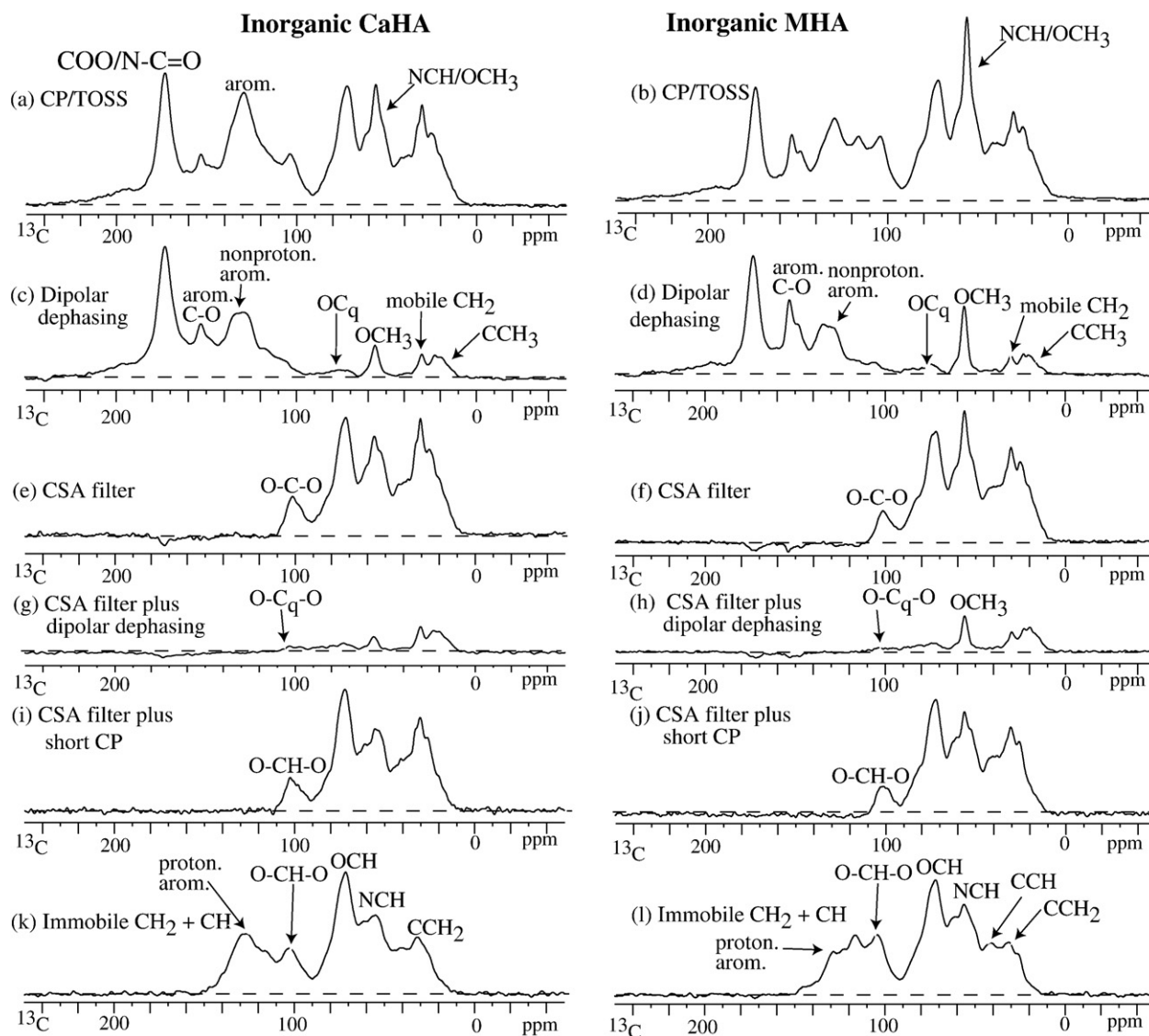
Fig. 3. Spectral editing for identification of functional groups in ^{13}C NMR of inorganic calcium HA (a, c, e, g, i, and k) and mobile HA (b, d, f, h, j, and l). (a, b) Unselective CP/TOSS spectra for reference, with a contact time of 1 ms at a spinning speed of 6.5 kHz. (c, d) Corresponding dipolar-dephased CP/TOSS spectra showing nonprotonated carbons and mobile segments such as CH$_3$, acquired after 40-μs of decoupling gated off. (e, f) Selection of sp^3-hybridized carbon signals by a chemical-shift anisotropy filter, which in particular identifies OCO carbons, near 102 ppm, which are typical of sugar rings. CSA filter time: 35 μs, other parameters as in (a). (g, h) Corresponding selection of nonprotonated or mobile sp^3-hybridized carbon signals by a 35-μs CSA filter and 40-μs dipolar dephasing, which in particular identifies OC$_q$O carbons near 100 ppm. (i, j) Complementary selection of protonated sp^3-hybridized carbons, in particular OCHO around 102 ppm, by a 35-μs CSA filter and short, 50-μs CP. (k, l) Protonated carbons only, respectively. Recycle delays for all spectra were 1 s. Number of scans: 1536 for all spectra except 4096 for (a); 1322 for (b).

combination of this CSA filter technique with short CP (50 μs) results in subspectra of protonated alkyl carbons (Fig. 3(i) and (j)). In particular, they show a clear band of protonated O–CH–O moieties near 100 ppm. It is clear that the OCO is dominantly protonated for both the MHA and CaHA. Fig. 3(k) and (l) show the spectra with immobile CH plus CH$_2$ and a small CH$_3$. They show protonated aromatics, protonated anomerics, OCH, NCH, CCH, CCH$_2$, and also CH$_3$. The environments of protonated aromatics are significantly different between the MHA and CaHA. In the MHA (Fig. 3(l)), two bands are clearly observed around 115 and 130 ppm in the MHA and only one broad band is observed around 130 ppm in the CaHA, suggesting greater diversitiy of lignin residues in the MHA.

In order to further confirm the proposed differences between MHA and CaHA structures, Fig. 4 displays the ^{1}H–^{13}C Hetcor spectrum of inorganic MHA and inorganic CaHA with 0.5 ms of LGCP, which shows correlation peaks for one- and two-bond distances. Also the proton cross sections at the indicated ^{13}C chemical shifts were extracted to help identify the connectivities of different functional groups. At the ^{13}C chemical shift

of 172 ppm, the significant contribution is from NCH or OCH protons resonating at the ^{1}H chemical shift of 4.5 ppm. The contribution from HNC=O (amide) carbons, which are two bonds from the NH protons resonating at the ^{1}H chemical shift of 8.5 ppm, is also observed. There are also proton signals from COOH, which resonates around 11 pm in the ^{1}H dimension. At the ^{13}C chemical shift of 152 ppm, the dominant contribution is from aromatic C–O carbons, and it has a shoulder from protons of OCH$_3$ protons. Correspondingly, the cross section at the ^{13}C chemical shift of 55 ppm displays a shoulder at ca. 7 ppm from the aromatic protons. These cross peaks indicate the existence of lignin residues in both samples. They are more prominent in the MHA than in the CaHA, indicating more lignin residues in the MHA. The cross section at the ^{13}C chemical shift of 128 ppm of both MHA and CaHA, a contribution from primarily the nonprotonated aromatic C (Fig. 2(h)), shows a shoulder which could be from CCH. In contrast, the cross section at the ^{13}C chemical shift of 116 ppm of the MHA, the contribution from primarily protonated aromatic carbons (Fig. 2(h)), does not possess such a proton signal. The CCH (41 ppm cross section) and CCH$_2$ (30 ppm cross section) groups both
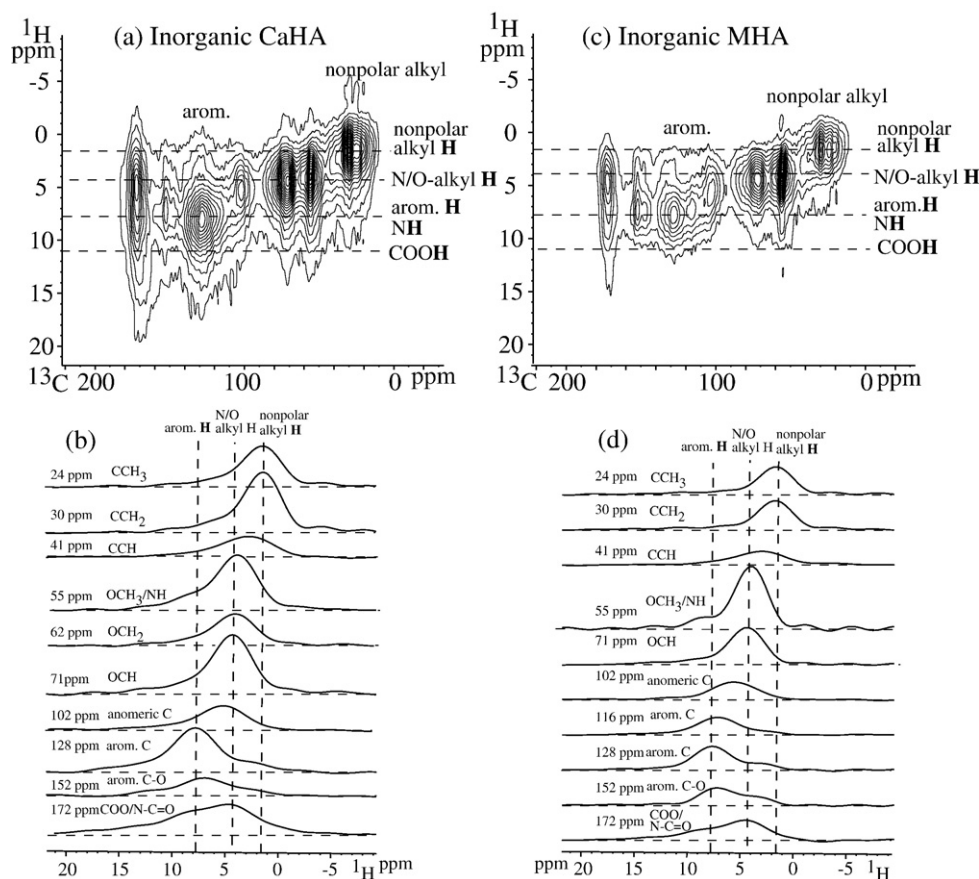
Fig. 4. (a) 2D HETCOR spectra with 0.5-ms LGCP of inorganic CaHA and (b) Cross sections from spectrum (a) at the indicated ^{13}C chemical shifts: 24, 30, 41, 55, 62, 71, 102, 128, 152, and 172 ppm. (c) 2D HETCOR spectra with 0.5-ms LGCP of inorganic MHA. (d) Cross sections from spectrum (c) at ^{13}C chemical shifts indicated: 24, 30, 41, 55, 71, 102, 115, 128, 152, and 172 ppm. Recycle delays for both spectra were 1 s, number of scans 256 per t_1 increment, and 96 t_1 increments.

show signals of NCH or OCH protons resonating at 4.5 ppm. The fact that there are two aromatic bands in the MHA but only one in the CaHA can be more clearly observed in their 2D HETCOR spectra by resolving the peaks not only in the ^{13}C but also in the ^{1}H dimension.

3.4. FT-IR analysis

The FT-IR spectra of the six humic samples were representative of soil humic fractions (Fig. 5). The FT-IR spectra did not differ noticeably among fertilization treatments. However, spectral differences were observed between MHA and CaHA fractions. The FT-IR bands are interpreted based on previous reports with humic substances (Francioso et al., 1996; Agnelli et al., 2000; Olk et al., 2000; Tan, 2003; He et al., 2006b).

In the MHA spectra (Fig. 5(a)), the broad band around 3354 cm^{-1} can be assigned to O–H and N–H stretching, and the bands at about 2935 and 2854 cm^{-1} to asymmetric and symmetric C–H stretching motions of aliphatic groups. In the CaHA spectra (Fig. 5(b)), these bands became less intense and shifted toward lower wave numbers, appearing at 3315, 2933, and 2847 cm^{-1}, respectively. The band at 1720 cm^{-1} was generally due to C=O stretching of COOH and other carbonyl groups. This band was merely a shoulder in the MHA but a true peak in the spectra of the CaHA (Fig. 5(b)). The two sharp bands or shoulders at 1655–1590 cm^{-1} were due to aromatic C=C vibrations, symmetric stretching of COO$^-$ groups, and H-bonded C=O of conjugated ketones. The bands at 1655 cm^{-1} were stronger in the MHA spectra than in the CaHA spectra. Spectral differences were also observed in the 1542–1330 cm^{-1} region. The bands at 1508, 1454, 1421, and 1330 were distinct in the MHA spectra but were weaker in

the CaHA spectra. These bands are preferentially assigned to aliphatic C–H, and O–H deformation, C–O stretching of phenolic OH, and COO$^-$ antisymmetrical stretching. The broad band around 1224 cm^{-1}, appearing in both spectra, is attributed to C–O stretching and O–H deformation of carboxyls, phenols, and aromatic ethers. The band at 1125 cm^{-1}, pronounced in the MHA spectra, is attributed to the C–O vibration of alcohol and ether groups (Olk et al., 2000). The bands at 1075–1028 cm^{-1} have frequently been assigned to alcoholic and polysaccharide C–O stretching or SiO$_2$ impurity (Giusquiani et al., 1998; Agnelli et al., 2000; Olk et al., 2000; Tan, 2003). We would like to attribute them, at least partially, to phosphate groups, as they correspond to the abundance of P in the samples (Francioso et al., 1996; He et al., 2006b). Accordingly, total P was more abundant in the MHA fractions of some rice soils than in the corresponding CaHA fractions (Mahieu et al., 2000).

The profiles of all spectra support two main observations. First, the multiple bands that represent aliphatic groups are more distinct in the MHA spectra than the CaHA spectra. Second, these bands shift towards lower wave numbers in the CaHA spectra. These two observations suggest that the MHA fractions were more aliphatic and had a greater diversity of aliphatic fractions than did the CaHA fractions. This result is consistent with the findings of DP/MAS NMR, which also showed more aliphatics in the MHA than the CaHA (Table 2).

4. Discussion

The NMR and FT-IR results indicate significant structural differences between the MHA and CaHA fractions. By comparison, fertilizer treatments led to more modest differences in humic chemistry.
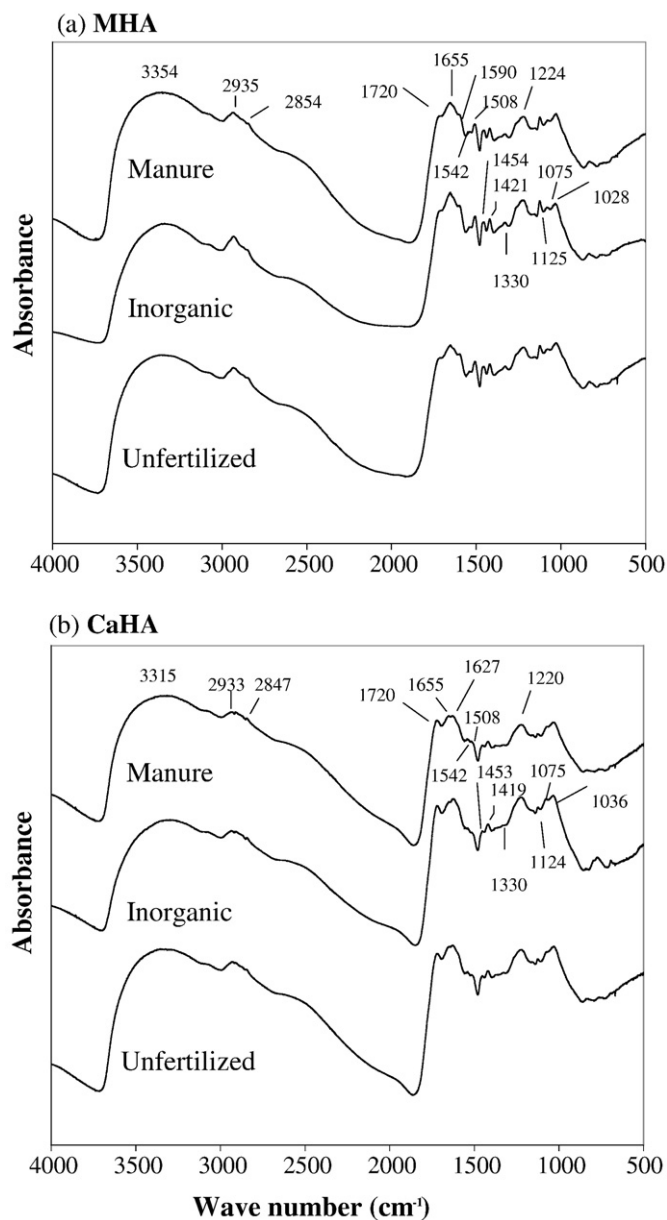
Fig. 5. FT-IR spectra of the (a) MHA and (b) CaHA fractions from three fertilizer treatments. Band assignments are indicated in the text.

4.1. Influence of fertilization on the structures of soil organic matter

The key issue in this study was whether amendment of animal manuring for 5 years affected the chemical nature of SOM in a manner that could account for the observed improved availability of soil N and P to a corn crop. The NMR analyses associated manure application with several structural changes, while FT-IR analysis detected no effect of the manuring. Of direct relevance to the soil N supply, in selective NMR spectra the CaHA was shown to have a ~13% increase in NCH groups due to manure application, indicating greater abundance of peptide N. This increase, and the corresponding increase in NCO groups, was partially masked in unselective spectra by a decrease in OCH_3 and COO groups associated with the aromatic component, whose signals overlap with the NCH and NC=O resonances, respectively, demonstrating the improved accuracy of the selective NMR techniques. Interestingly, this peptide enrichment was found in the older of the two humic fractions, the CaHA, in agreement with the total N concentrations of these two fractions. In previous studies, the chemical nature of the MHA was more responsive to recent crop

management than that of the CaHA (Olk, 2006). Because the MHA is inherently N-rich, we speculate that the most apparent response of the MHA fraction to manuring in this study was increased quantity (55% increase for MHA, 32% increase for CaHA), while an N enrichment was more apparent in the typically N-depleted CaHA fraction.

Further, NMR analysis of the CaHA showed that manure application resulted in a pronounced enrichment of nonpolar alkyl functional groups. It was attributed mostly to greater prominence of nonpolar alkyl CH_2, which we believe likely represents fatty acids. Their accumulation could have included fatty acids that occur naturally in animal manure (Mao et al., 2008; Jandl et al., 2005) or cellular residues from increased soil microbial growth that is often associated with animal manure application (Ndayegamiye and Côté, 1989; Albiach et al., 2000; Fliebbach et al., 2007).

The NMR results also indicated decreased abundance of aromatic C in the manure CaHA. This change does not merely reflect a dilution by the accumulating nonpolar alkyl compounds, because the spectral proportions of the other functional groups did not decrease in parallel with the aromatic compounds. At this time, we have no clear explanation for the decreased aromatic nature with manuring. It might result from enhanced degradation of ligneous matter by the increased microbial activity noted above, whether the microorganisms were native to the soil and were stimulated by input of manure-C or they were added to the soil as part of the manure.

Hence animal manuring appears to have caused a shift in the content of the CaHA fraction toward nonpolar alkyl compounds, which is consistent with the review by Senesi et al. (2007) for manure and other organic amendments and with the accumulation of fatty acids described by Jandl et al. (2005). Our finding of enriched NCH groups, i.e. peptide compounds, is consistent with the amino-N enrichment attributed to manuring by Campbell et al. (1986) and Brunetti et al. (2007).

Although the chemical nature of the manure used in this field study was not determined, a relatively aliphatic, labile nature of fresh (i.e. not composted) manure components has been broadly presumed in other studies, based on both chemical characterization (Senesi et al., 2007) and the rapid release of manure-bound nutrients (Sims and Wolf, 1994; Bouldin and Klausner, 1998; Sharpley et al., 1998). We hypothesize that a relatively aliphatic, labile nature of the manure that was added to this Nebraska field enriched the SOM in labile compounds that readily mineralized, leading to a steady nutrient supply to the corn crop and possibly improved soil physical properties. Some or all of these outcomes likely contributed to the improved corn yield noted for portions of the field. To build on this study, future work will further characterize these humic fractions together with humic fractions from the low-C portions of the field for two soil sampling times near the beginning and end of one growing season. Humic, whole soil, and soil aggregate samples will be analyzed chromatographically for amino compounds, fatty acids, and other biochemical compounds.

4.2. Detailed structural difference between MHA and CaHA

Detailed structural comparison of the two humic fractions demonstrated that MHA contains more lignin while CaHA has more aromatics. In the 90–162 ppm region, the NMR spectra of the CaHA have three bands (102, 128, and 152 ppm) but the MHA spectra have four (102, 116, 128, 152 ppm) (Fig. 2(a) and (g)). The MHA spectra were more similar to typical spectra of lignin-rich organic matter than were the CaHA spectra (Schmidt-Rohr et al., 2004). Also, the band around 152 ppm for MHA is actually composed of one peak at 152 ppm and another at 148 ppm. Amalfitano et al. (1992) assigned the 148 ppm peak to syringyl phenols and the 152 ppm peak to guaiacyl phenols. These two phenol types are dominant components of lignin. In addition, the NMR analysis with CP/TOSS spectra after 40-μs of dipolar dephasing indicated an enrichment of lignin-derived compounds in the MHA, which is demonstrated by more OCH_3 and more aromatic C–O.

Analyses by both NMR and FT-IR indicated a greater aliphatic nature of the MHA fraction compared to the CaHA fraction. The MHA fraction appeared to have both larger quantities (NMR) and greater diversity (FT-IR) of aliphatic compounds. These results are consistent with previous comparisons of the MHA and CaHA fractions in (i) a California cotton soil (Olk et al., 1995) that involved ^{13}C NMR, and (ii) Philippine rice soils that involved ^{13}C NMR, cupric oxide extraction for lignin-derived phenols (Olk et al., 1996, 1998), FT-IR (Olk et al., 1999, 2000) and pyrolysis–gas chromatography–mass spectrometry (Olk et al., 2002). Both the greater aliphatic nature and phenol enrichment of the MHA fraction suggest the presence of more labile, less decomposed materials, which is consistent with the operational definitions of the MHA fraction as being unbound, relatively young material and the CaHA fraction as humic material that has been chemically stabilized against degradation by soil Ca (Olk, 2006).

The chemical structure of the MHA fraction in this study partially resembles that of the MHA fraction from a triple-cropped tropical rice soil (Schmidt-Rohr et al., 2004), apparently due to significant contributions from lignin that has undergone limited humification. During intensive cropping, the rice soil was submerged for 11 months annually for 30 years. Thus, the MHA of that soil contained significantly less decomposed organic materials, especially lignin which accounted for at least 45% of all carbons. The spectral-editing techniques (Fig. 3) indicated that similar to the MHA from the triple-cropped rice soil, the MHA of the Nebraska soil also contained lignin derivatives as demonstrated above, and also peptides which are supported by the existence of N–C=O and NCH, and carbohydrates which was demonstrated by the existence of anomerics and OC. The structure of the aromatics in the CaHA has some characteristics similar to those of humic acids from two Mollisols in Iowa (Mao et al., 2007c), which were dominated by aromatics, possibly from black carbons. A more degraded, humified state of the CaHA fraction compared to the MHA fraction was indicated by the enrichment of COO/N–C=O in the CaHA fraction: carboxyl groups are thought to accumulate in humic substances with increasing humification (Stevenson, 1994).

4.3. NMR versus FT-IR

An effect of a 5-year manure application on the chemical nature of the MHA and CaHA fractions was observed solely by NMR, namely the more aliphatic and peptide-rich nature and less aromatic nature with manure addition for CaHA. Likewise, Brunetti et al. (2007) also found that NMR detected effects of manure on humic chemistry whereas FT-IR did not. Both analyses detected a more aliphatic nature of the MHA compared to the CaHA, but the NMR analysis enabled the calculation of relative abundances, while FT-IR did not enable quantitative comparison. The NMR analysis also detected the accumulation of lignin-derived compounds in the MHA compared to the CaHA.

Acknowledgments

References

Agnelli, A., Celi, L., Degl'Innocenti, A., Corti, G., Ugolini, F.C., 2000. Chemical and spectroscopic characterization of the humic substances from sandstone-derived rock fragments. Soil Sci. 165, 314–327.

Albiach, R., Canet, R., Pomares, F., Ingelmo, F., 2000. Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil. Bioresour. Technol. 75, 43–48.

Amalfitano, C., Pignalosa, V., Auriemma, L., Ramunni, A., 1992. The contribution of lignin to the composition of humic acids from a wheat-straw amended soil during 3 years of incubation in pots. J. Soil Sci. 43, 495–504.

Bouldin, D.R., Klausner, S.D., 1998. Managing nutrients in manure: general principles and applications to dairy manure in New York. In: Hatfield, J.L., Stewart, B.A. (Eds.), Animal Waste Utilization: Effective Use of Manure as a Soil Resource. Lewis Publishers, Boca Raton, FL, pp. 65–88.

Brunetti, G., Plaza, C., Clapp, C.E., Senesi, N., 2007. Compositional and functional features of humic acids from organic amendments and amended soils in Minnesota, USA. Soil Biol. Biochem. 39, 1355–1365.

Campbell, C.A., Schnitzer, M., Stewart, J.W.B., Biederbeck, V.O., Selles, F., 1986. Effect of manure and P fertilizer on properties of a Black Chernozem in southern Saskatchewan. Can. J. Soil Sci. 66, 601–613.

Dixon, W.T., 1982. NMR spectra in spinning sidebands (TOSS). J. Chem. Phys. 77, 1800–1809.

Fan, T.W.-M., Higashi, R.M., Lane, A.N., 2000. Chemical characterization of a chelator-treated soil humate by solution-state multinuclear two-dimensional NMR with FTIR and pyrolysis-GCMS. Environ. Sci. Technol. 34, 1636–1646.

Fliebbach, A., Oberholzer, H.-R., Gunst, L., Mäder, P., 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agric. Ecosyst. Environ. 118, 273–284.

Francioso, O., Sanchez-Cortes, S., Tugnoli, V., Ciavatta, C., Sitti, L., Gessa, C., 1996. Infrared, Raman, and nuclear magnetic resonance (1H, 13C, and 31P) spectroscopy in the study of fractions of peat humic acids. Appl. Spectrosc. 50, 1165–1174.

Giusquiani, P.L., Concezzi, L., Busibelli, M., Macchioni, A., 1998. Fate of pig sludge liquid fraction in calcareous soil: agricultural and environmental implications. J. Environ. Qual. 27, 364–371.

Hatfield, J.L., Stewart, B.A. (Eds.), 1998. Animal Waste Utilization: Effective Use of Manure as a Soil Resource. Lewis Publishers, Boca Raton, FL.

He, Z., Honeycutt, C.W., Zhang, T., Bertsch, P.M., 2006a. Preparation and FT-IR characterization of metal phytate compounds. J. Environ. Qual. 35, 1319–1328.

He, Z., Ohno, T., Cade-Menun, B.J., Erich, M.S., Honeycutt, C.W., 2006b. Spectral and chemical characterization of phosphates associated with humic substances. Soil Sci. Soc. Am. J. 70, 1741–1751.

He, Z., Honeycutt, C.W., Xing, B., McDowell, R.W., Pellechia, P.J., Zhang, T., 2007. Solid state Fourier transform infrared and 31P nuclear magnetic resonance spectral features of phosphate compounds. Soil Sci. 172, 501–515.

Hu, W.-G., Mao, J.-D., Xing, B., Schmidt-Rohr, K., 2000. Poly(methylene) crystallites in humic substances detected by nuclear magnetic resonance. Environ. Sci. Technol. 34, 530–534.

Hergert, G.B., Ferguson, R.B., Shapiro, C.A., 1995. Fertilizer suggestions for corn. NebGuide G74-174-A, Cooperative Extension, Universitiy of Nebraska.

Jandl, G., Leinweber, P., Schulten, H.-R., Ekschmitt, K., 2005. Contribution of primary organic matter to the fatty acid pool in agricultural soils. Soil Biol. Biochem. 37, 1033–1041.

Legorreta-Padilla, F.d.J., 2005. The impact of maize and soybean cropping systems on carbon and nitrogen dynamics in soil organic matter. Ph.D. thesis, University of Nebraska, Lincoln.

Levin, E.M., Bud'ko, S.L., Mao, J.-D., Huang, Y., Schmidt-Rohr, K., 2007. Effect of magnetic particles on NMR spectra of Murchison meteorite organic matter and a polymer-based model system. Solid State NMR 31, 63–71.

Mahieu, N., Olk, D.C., Randall, E.W., 2000. Analysis of phosphorus in two humic acid fractions of intensively cropped lowland rice soils by ^{31}P-NMR. Eur. J. Soil Sci. 51, 391–402.

Mahieu, N., Olk, D.C., Randall, E.W., 2002. Multinuclear magnetic resonance analysis of two humic acid fractions from lowland rice soils. J. Environ. Qual. 31, 421–430.

Mao, J.-D., Schmidt-Rohr, K., 2003. Long-range C–H dipolar dephasing in solid-state NMR, and its use for spectral selection of fused aromatic rings. J. Magn. Reson. 162, 217–227.

Mao, J.-D., Schmidt-Rohr, K., 2004a. Accurate quantification of aromaticity and unprotonated aromatic carbon fraction in natural organic matter by 13C solid-state NMR. Environ. Sci. Technol. 38, 2680–2684.

Mao, J.-D., Schmidt-Rohr, K., 2004b. Separation of acetal or ketal O–C–O 13C NMR signals from aromatic-carbon bands by a chemical-shift-anisotropy filter. Solid State NMR 26, 36–45.

Mao, J.-D., Schmidt-Rohr, K., 2005. Methylene spectral editing in solid-state NMR by three-spin coherence selection. J. Magn. Reson. 176, 1–6.

Mao, J.-D., Schmidt-Rohr, K., 2006. Absence of mobile carbohydrate domains in dry humic substances proven by NMR, and implications for organic-contaminant sorption. Environ. Sci. Technol. 40, 1751–1756.

Mao, J.-D., Hu, W., Schmidt-Rohr, K., Davies, G., Ghabbour, E.A., Xing, B., 2000. Quantitative characterization of humic substances with solid state NMR. Soil Sci. Soc. Am. J. 64, 873–884.

Mao, J.-D., Hundal, L.S., Schmidt-Rohr, K., Thompson, M.L., 2003. Nuclear magnetic resonance and diffuse-reflectance infrared Fourier-transform spectroscopy of sludge-derived biocolloidal organic matter. Environ. Sci. Technol. 37, 1751–1757.

Mao, J.-D., Holtman, K.M., Scott, J.T., Kadla, J.F., Schmidt-Rohr, K., 2006. Differences between lignin in unprocessed wood, milled wood, mutant wood, and extracted lignin detected by 13C Solid-State NMR. J. Agric. Food Chem. 54, 9677–9686.

Mao, J.-D., Tremblay, L., Gagné, J.-P., Kohl, S., Rice, J., Schmidt-Rohr, K., 2007a. Humic acids from particulate organic matter in the Saguenay Fjord and the St. Lawrence Estuary investigated by advanced solid-state NMR. Geochim. Cosmochim. Acta 71, 5483–5499.

Mao, J.-D., Cory, R.M., McKnight, D.M., Schmidt-Rohr, K., 2007b. Characterization of a nitrogen-rich fulvic acid and its precursor algae by solid-state NMR. Org. Geochem. 38, 1277–1292.

Mao, J.-D., Fang, X., Schmidt-Rohr, K., Carmo, A.M., Hundal, L.S., Thompson, M.L., 2007c. Molecular-scale heterogeneity of humic acid in particle-size fractions of two Iowa soils. Geoderma 140, 17–29.

Mao, J.-D., Ajakaiye, A., Lan, Y., Olk, D.C., Ceballos, M., Zhang, T., Fan, M.Z., Forsberg, C.W., 2008. Chemical structures of manure from conventional and phytase transgenic pigs investigated by advanced solid-state NMR spectroscopy. J. Agric. Food Chem. 56, 2131–2138.

Ndayegamiye, A., Côté, D., 1989. Effect of long-term pig slurry and solid cattle manure application on soil chemical and biological properties. Can. J. Soil Sci. 69, 39–47.

Olk, D.C., 2006. A chemical fractionation for structure(function relations of soil organic matter in nutrient cycling. Soil Sci. Soc. Am. J. 70, 1013–1022.

Olk, D.C., Cassman, K.G., Fan, T.W.M., 1995. Characterization of two humic acid fractions from a calcareous vermiculitic soil: implications for the humification process. Geoderma 65, 195–208.

Olk, D.C., Cassman, K.G., Randall, E.W., Kinchesh, P., Sanger, L.J., Anderson, J.M., 1996. Changes in chemical properties of organic matter with intensified rice cropping in tropical lowland soil. Eur. J. Soil Sci. 47, 293–303.

Olk, D.C., Cassman, K.G., Mahieu, N., Randall, E.W., 1998. Conserved chemical properties of young humic acid fractions in tropical lowland soil under intensive irrigated rice cropping. Eur. J. Soil Sci. 49, 337–349.

Olk, D.C., Brunetti, G., Senesi, N., 1999. Organic matter in double-cropped lowland rice soils: Chemical and spectroscopic properties. Soil Sci. 164, 633–649.

Olk, D.C., Brunetti, G., Senesi, N., 2000. Decrease in humification of organic matter with intensified lowland rice cropping: a wet chemical and spectroscopic investigation. Soil Sci. Soc. Am. J. 64, 1337–1347.

Olk, D.C., Dancel, M.C., Moscoso, E., Jimenez, R.R., Dayrit, F.M., 2002. Accumulation of lignin residues in organic matter fractions of lowland rice soils: a pyrolysis-GC-MS study. Soil Sci. 167, 590–606.

Olk, D.C., Samson, M.I., Gapas, P., 2007. Inhibition of nitrogen mineralization in young humic fractions by anaerobic decomposition of rice crop residues. Eur. J. Soil Sci. 58, 270–281.

Olsen, S.R., 1986. The role of organic matter and ammonium in producing high corn yields. In: Chen, Y., Avnimelech, Y. (Eds.), The Role of Organic Matter in Modern Agriculture. Martinus Nijhoff Publishers, Dordrecht, pp. 29–54.

Paul, E.A., Paustian, K., Elliott, E.T., Cole, C.V., 1997. Soil Organic Matter in Temperate Agroecosystems: Long-term Experiments in North America. CRC Press, Boca Raton, FL.

Plaza, C., Senesi, N., García-Gil, J.C., Brunetti, G., D'Orazio, V., Polo, A., 2002. Effects of pig slurry application on soils and soil humic acids. J. Agric. Food Chem. 50, 4867–4874.

Preston, C.M., 1996. Application of NMR to soil organic matter analysis (history and prospects). Soil Sci. 161, 144–166.

Randall, E.W., Mahieu, N., Powlson, D.S., Christensen, B.T., 1995. Fertilization effects on organic matter in physically fractionated soils as studied by C-13 NMR: results from two long-term field experiments. Eur. J. Soil Sci. 46, (557–565.

Schmidt-Rohr, K., Mao, J.-D., 2002a. Efficient CH-group selection and identification in ^{13}C Solid-state NMR by dipolar DEPT and ^{1}H chemical-shift filtering. J. Am. Chem. Soc. 124, 13938–13948.

Schmidt-Rohr, K., Mao, J.-D., 2002b. Selective dephasing of NH and OH proton magnetization based on ^{1}H anisotropy recoupling. J. Magn. Reson. 157, 210–217.

Schmidt-Rohr, K., Mao, J.-D., 2002c. Selective observation of nitrogen-bonded carbons in solid state NMR by saturation-pulse induced dipolar exchange with recoupling. Chem. Phys. Lett. 359, 403–411.

Schmidt-Rohr, K., Mao, J.-D., Olk, D.C., 2004. Nitrogen-bonded aromatics in soil organic matter and their implications for a yield decline in intensive rice cropping. PNAS, USA, 101, 6351–6354.

Senesi, N., Plaza, C., Brunetti, G., Polo, A., 2007. A comparative survey of recent results on humic-like fractions in organic amendments and effects on native soil humic substances. Soil Biol. Biochem. 39, 1244–1262.

Sharpley, A., Meisinger, J.J., Breeuwsma, A., Sims, J.T., Daniel, T.C., Schepers, J.S., 1998. Impacts of animal manure management on ground and surface water quality. In: Hatfield, J.L., Stewart, B.A. (Eds.), Animal Waste Utilization: Effective Use of Manure as a Soil Resource. Lewis Publishers, Boca Raton, FL, pp. 173–242.

Sims, J.T., Wolf, D.C., 1994. Poultry waste management: agricultural and environmental issues. Adv. Agron. 52, 1–83.

Stevenson, F.J., 1994. Humus Chemistry: Genesis, Composition, Reactions, 2nd ed. Wiley, New York.

Swift, R.S., 1996. Organic matter characterization. In: Sparks, D.L., et al. (Ed.), Methods of Soil Analysis. Part 3 Chemical Methods. Soil Science Society of America, Madison, WI, pp. 1011–1069.

Tan, K.H., 2003. Humic Matter in Soil and the Environment. Marcel Dekker, Inc., New York, N.Y.

Van Faassen, H.G., Van Dijk, H., 1987. Manure as a source of nitrogen and phosphorus in soils. In: Van Der Meer, H.G., et al. (Ed.), Animal Manure on Grassland and Fodder Crops. Fertilizer or Waste? Martinus Nijhoff, Dordrecht, Netherlands, pp. 27–45.

Wander, M.M., Traina, S.J., 1996. Organic matter fractions from organically and conventionally managed soils: II. Characterization of composition. Soil Sci. Soc. Am. J. 60, 1087–1094.